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Applying Models of Targeted Drug Delivery to Gene Delivery

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Abstract—**Gene delivery requires targeted delivery systems. Exploratory simulations using models of targeted drug delivery helps one assess the worthiness of such systems, and helps quantify the expected therapeutic benefits of the systems. The drug targeting index (DTI), a ratio of availabilities, is a measure of pharmacokinetic benefit of the delivery device, based on a combination of a physiologically-based pharmacokinetic model and a single pharmacodynamic E_{max} model. Pharmacodynamic outcomes are quantified by the degree of separation between the dose-response and dose-toxicity curves (SRT). Simulations are undertaken to investigate the potential linkage of DTI and SRT, a pharmacodynamic outcome. A significant positive linear relationship is found between the DTI and SRT. The relationship can be translated into a minimum pharmacokinetic requirement that can be used to guide making decisions regarding whether or not further pursue the development of a candidate gene-delivery device as a therapeutic agent.**

Key Words—**Targeted drug delivery, gene delivery, population pharmacokinetics, pharmacodynamic, modeling, simulation, systems biology**

I. Introduction

Gene delivery faces intrinsic difficulties: genes or gene-interfering agents, in form of plasmid, single-stranded or double stranded RNA or DNA, or short oligonucleotides, cannot readily access their target response sites – the nuclei of a *specific* cell group. Giving a huge dose of free RNA or DNA, and hoping that enough will distribute into target tissues does not work. On one hand, free RNA and DNA cannot freely permeate cellular membranes. On the other hand, free RNA and DNA are rapidly degraded in the circulation. They therefore must be protected by some carrier, or be chemically modified to make them resistant to degradation. Hence, there is a need for *targeting*. Gene delivery to a specific cell group usually requires targeted delivery devices, such as cationic liposomes or viral vectors. Engineering an efficient delivery system is a must to ensure proper gene delivery, and in turn, expected efficacy. Hence, it is prudent to use models of targeted drug delivery to analyze any candidate gene delivery system and thereby help inform the decision making process involved in development as a therapeutic agent. Failure to identify poor candidate delivery systems will result in a lengthy yet unsuccessful development program, not to mention the burden of the financial loss. Better-informed assessment is critical early in the drug development process.

This study applies a physiologically-based population pharmacokinetic model of targeted drug delivery to gene delivery. The model used [1], provides a pharmacokinetic assessment of the targeting device, namely, the drug targeting index, DTI, which is a ratio of availability of drug in the target response compartment to its corresponding availability at the sites that give rise to toxicity. DTI is conveniently taken as the ratio of area under the concentration-time curves (AUC) or the ratio of drug exposures at the two sites. In the simplest sense, if one assumes steady state delivery, the DTI equals the ratio of the level of drug or transcriptionally active genes at the response site(s) to the corresponding level at the sites of toxicity. It will be shown in this report, that such pharmacokinetic assessments can directly reflect pharmacodynamic consequences and thus can be a good estimate of expected performance in terms of increasing the separation between of dose-response and dose-toxicity curves. These simulation experiments are population-based so as to provide some insight into expected inter-subject variability. That variability can have a have a decisive impact on how likely it is that the device under study functions as intended.

II. Methods

A. Simulations

The physiologically-based pharmacokinetic model is a simplification of that described earlier [1]. Briefly, the body is represented as two physiologic compartments. For the sake of discussions of gene delivery, the response site is taken as the specific groups of cell to which genes need to be delivered in order to generate the desired pharmacological response. The site of toxicity is conservatively taken as everywhere else in the body. DTI is the ratio of active levels at these two sites. A set of differential equations is solved using the steady state assumption to give concentrations at the two sites in terms of dose (D), blood flows (to response site, Q_r, and to the site of toxicity, Q_t), extractions at each site (at E_r and E_t), delivery to each site (to response site, F_r, and to the site of toxicity, F_t) and fu is the uncompromised fraction (for a drug, bound)—the fraction available to generate gene products. The values of C_r and C_t are given by the following equations.

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$$C_r = \frac{D \times (1 - fuE_r) \times \left[\left(Q_r + fuQ_t E_t\right)\left(\frac{F_r}{Q_r}\right) + (1 - fuE_t) \times F_t \right]}{fu \times (Q_r E_r + Q_t E_t)}$$

$$C_t = \frac{D \times (1 - fuE_t) \times \left[\left(Q_t + fuQ_r E_r\right)\left(\frac{F_t}{Q_t}\right) + (1 - fuE_r) \times F_r \right]}{fu \times (Q_r E_r + Q_t E_t)}$$

As a first approximation, a simplistic pharmacodynamic model is used. Briefly, the pharmacological effects follow a single E_{max} model for the uncompromised level of active species at the response site. The uncompromised fraction (unbound fraction for a drug) is used because Tachibana *et al* [2] observed that only a small fraction of delivered plasmid can be transported into the nucleus to become available for transcription or interference. Although the pharmacodynamic model oversimplifies the complicated sub-cellular processes, it allows assessment of the overall pharmacodynamic outcomes of the targeted delivery of genes or a drug given a simple steady state concentration. The parameters are apparent maximum effects (of response, P_{max}, and of toxicity, T_{max}) and apparent concentrations at which half-maximal effect is seen (of response, $C_{P,50}$, and of toxicity, $C_{T,50}$). The following equations are used.

$$\text{Response} = \frac{P_{max} \times fuC_r}{fuC_r + C_{P,50}} \qquad \text{Toxicity} = \frac{T_{max} \times fuC_t}{fuC_t + C_{T,50}}$$

The simulations are run using a population of N = 1000 hypothetical subjects. The values of each subject's individual variable are sampled from either a normal or a beta distribution having a specified mean and coefficient of variation. The key summary statistics that give rise to the individual parameters of the population are listed in Table 1.

To quantify pharmacodynamic benefit, each subject is simulated to receive 14 doses, from 0.001 to 20, so as to follow the entire dose-response and dose-toxicity curves. The total separation between the response and toxicity curves (SRT) is a pharmacodynamic assessment of the drug. Graphically, it is the area between the response and toxicity curves, shown as the shaded area in Fig 1. In the study, it is approximated by summing, over all doses, the difference between response and toxicity at each dose: $\sum_{\text{Dose}}[(\text{Response - Toxicity})]$.

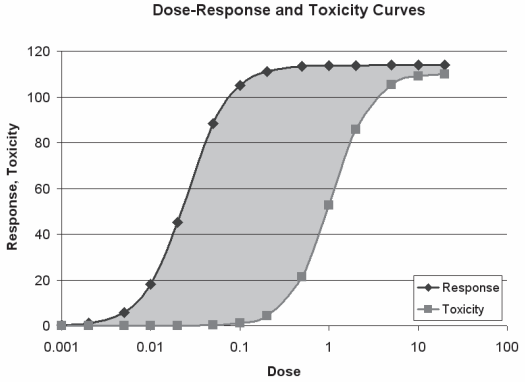

Fig. 1. Dose-response and dose-toxicity curves. The shaded area is the total separation of response and toxicity (SRT), and is a measure of pharmacodynamic benefit.

TABLE 1:
SUMMARY STATISTICS OF THE POPULATION N = 1000

Variable	Mean	Median	Std Dev
Blood flow to response site (Q_r)	5.05	5.05	3.00
Blood flow to toxic site (Q_t)	4978	4974	495
Extraction at response site (E_r)	0.900	0.987	0.177
Extraction at toxic site (E_t)	0.896	0.990	0.182
Delivery to response site (F_r)	0.0990	0.0972	0.0199
Delivery to toxic site (F_t)	0.901	0.903	0.0199
Uncompromised fraction (fu)	0.100	0.0768	0.0923
Apparent maximum response (P_{max})	100	101	20.4
Apparent maximum toxicity (T_{max})	100	101	19.6
Apparent concentration for half-maximal response ($C_{P,50}$)	9.89e-5	9.89e-5	1.97e-5
Apparent concentration for half-maximal toxicity ($C_{T,50}$)	2.00e-3	1.99e-3	4.02e-4

B. Derivation

A single E_{max} expression can represent multiple subcellular processes, if a single E_{max} model can represent each of them, in turn.

$$\text{Suppose} \quad B = \frac{A_{max} \times A}{A_{50} + A} \quad \text{and} \quad C = \frac{B_{max} \times B}{B_{50} + B}$$

$$C = \frac{B_{max} \times \dfrac{A_{max} \times \dfrac{A}{A_{50}}}{1 + \dfrac{A}{A_{50}}}}{B_{50} + \dfrac{A_{max} \times \dfrac{A}{A_{50}}}{1 + \dfrac{A}{A_{50}}}} = \frac{\dfrac{B_{max} \times A_{max}}{(B_{50} + A_{max})} \times A}{\dfrac{B_{50} \times A_{50}}{(B_{50} + A_{max})} + A}$$

is a E_{max} model in A.

The effect of exposure is estimated by the area under the effect-time curve (AUE), integrated from time zero to infinity. Effect is represented using the above as an E_{max} model. Following a bolus dose, in which the concentration falls exponentially, AUE is derived as follows.

$$AUE = \int_0^{\infty} \frac{Emax \times C}{K + C} \mathrm{dt}$$

$$= \int_0^{\infty} \frac{Emax \times C_0 e^{-\lambda t}}{K + C_0 e^{-\lambda t}} \mathrm{dt}$$

$$= \frac{Emax}{\lambda} \ln\left(1 + \frac{C_0}{K}\right)$$

III. RESULTS

Fig. 2 depicts the relationship between the drug targeting index (DTI) and the separation between the response and toxicity curves (SRT). The pharmacokinetic assessment, i.e., the DTI, is linearly related to the pharmacodynamic assessment, the SRT. The significance of this relationship is that, given a favorable pharmacokinetic profile (e.g., high F_r/F_t in particular) and information supporting that the assumptions are reasonable, one can assert that the targeted drug will perform well in the population, pharmacodynamically, *without* having to actually test it in the population. The DTI, in turn, is a valuable assessment of worthiness of developing the candidate, targeted drug delivery system, as it provides estimates of the expected pharmacodynamic outcomes, *before* a clinical trial is run. A candidate drug having a too small a DTI can be confidently dropped from development for the reason that it is not likely to be clinically successful.

It is possible to estimate a minimum DTI requirement to be qualified for further development, if one has a sense of how much separation of response and toxicity is desired in the average patient. Suppose, it is *arbitrarily* chosen that a minimum separation of 25 units is desired if one assumes there is little toxicity associated for such a "low" dose, then it is a quarter of maximum transcriptional capacity of a gene, which can be a substantial amount. The results of simulations show that at the optimal dose (at which the maximum separation is achieved) one actually obtains only about 15% of the total achievable separation of about 167 units; that translates into a DTI of about 3.8. So, a candidate drug for which DTI = 3.8[1], can be expected to give a response-toxicity separation of 25 units, at the optimal dose in the average patient. Conversely, if a candidate is observed or estimated to have a DTI of 3.8, then a separation of 25 can be expected in the average patient. The relationship between SRT and DTI is thus translated into a guide for drug development decision making.

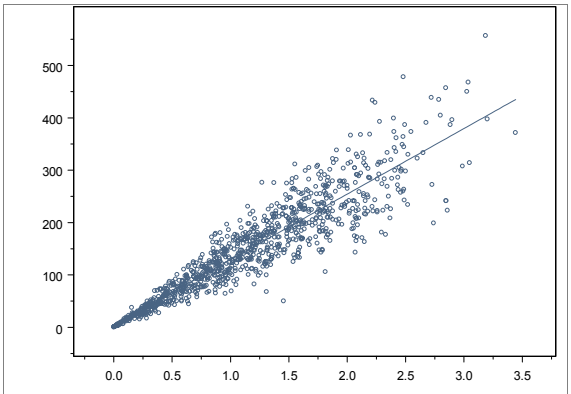

Fig. 2. The relationship between total SRT (y-axis) and logarithm of DTI (x-axis) is shown. The regression equation is y = 4.08+125*x., $R^2 = 0.85$. The intercept is not statistically significant, and the slope has a $p<0.0001$.

IV. DISCUSSION

It is of interest to contrast predictions made using the above over-simplified model with one that is more complicate. Rowland *et al*[3] proposed a subcompartment analysis at the response site to account for flux between subcompartments. The same concepts as above can be used to represent and simulate intracellular pharmacokinetics and nuclear transport. Kamiya *et al* [4] and Tachibana *et al* [2] also proposed simple zero-order or first-order intracellular models for intracellular trafficking of gene and gene expression. So, modeling the subcellular processes with these models will be the next step. These more sophisticated models would be expected to give a more detailed description of the intracellular processes, and should allow one to generate estimates of the most meaningful mechanistic rate constants that are need for effective delivery devices. However, early in a drug development program little is known about such properties. The above over-simplified model is therefore the logical alternative. It gives a simple assessment of the value or performance of the device, *without* extensive, costly experimentation or lengthy clinical trials, and therein lies the real advantage of its use.

The subcellcular processes are believed to be quite complicated. Is the above oversimplified pharmacodynamic E_{max} model, relating effect to intracellular cytoplasmic unbound levels adequate to aid decision making? That question will need to be answered for each individual case in light of available information. If an E_{max} model is adequate to represent each step in the process, then one global E_{max} model will be sufficient to represent the entire cascade of events, with the apparent E_{max} and C_{50} representing hybrids of the microscopic submodel parameters. The argument being made is consistent with the usual reported pharmacodynamic models in which a series of events, for example, a signal transduction cascade, is modeled by a single pharmacodynamic process. Consequently, a single global pharmacodynamic E_{max} model is used in this study to represent all of the subcellular processes. However, the parameters of such a model are empirical, not mechanistic, and cannot be used to explain or describe the subprocesses.

A limitation of the above approach is use of the steady state assumption. The simulation predictions are difficult to test experimentally. It is difficult to provide steady state gene delivery to cells *in vivo*. However, because the time scale for intracellular processes such as gene trafficking and gene expression are slow relative to distribution of drug or therapeutic agents into the cell, as observed by Tachibana et al [2], one can remove the steady-state assumption and, instead, approximate the input of gene as a bolus: assume that the therapeutic agent is given as a short infusion, until intracellular

[1] DTI = 3.8 means that the targeted delivery device successfully delivers approximately 4 fold higher levels to target cells, relative to an intravenous dose of untargeted therapeutic.

concentration of the loaded delivery device reaches its pharmacokinetic steady state, at which time the infusion is stopped. Now, the pharmacodynamic processes, which are slower, are effectively seeing the therapeutic input as a bolus into the cell. Both observing and simulating the effects, as a function of time, are complicated, thus rendering the simple model less valuable. What can be quantified, however, is an estimation of exposure to the effect, or the area under the effect-time curve (AUE.) To do so, it is assumed that the effect does not depend upon past exposure. Given therapeutic input as a bolus and having the effect represented by a single E_{max} model means that AUE is a function of C_0, the initial concentration and λ, first-order rate constant for elimination of the therapeutic at the effector site. There is still a positive relationship between the DTI, a ratio of C_0, and pharmacodynamic outcomes, quantified by ratio of AUEs. The exact relationship between the two, including consideration of the time lag and the dependence upon past exposure, will be the topic of further study.

Jiang *et al* reported successful gene delivery and expression with naked DNA injected intravenously into tail vein of the mouse without using a delivery device [5]. The limitation of this strategy is that the gene does not need to be delivered to a specific group of cells, and therefore non-specific uptake and expression of the gene by liver cells is sufficient to confer some therapeutic effects. The report also described a typical E_{max}-shaped dose-response effect of plasmid DNA on IL-10 expression in liver cells. Koh *et al* compared the delivery of naked IL-10 plasmid DNA versus to that delivered and protected by a biodegradable polymer [6]. The authors argued that the carrier protected the plasmid from being degraded in the circulation and, as a consequence, a larger effective amount of gene is delivered and is available for transcription. The study showed 3 to 5-fold increase in IL-10 expression by the delivery device, thereby validating the concept that improving the availability of gene for the effector increase the response. Finally, Liu *et al* compared the transfection efficiency of naked DNA versus that delivered by cationic lipids [7]. The studies measured radiolabeled intracellular DNA, and found that delivery by cationic lipids achieved several fold differences between different tissues, thereby supporting the argument above that a minimum fold difference in concentration between target and other tissue is achievable by such delivery devices.

One must not overlook the toxicity profile when developing a gene delivery device as a therapeutic agent. The common toxicity seen in clinical trials were immune reactions against the carrier vehicle, usually viral vectors, inflammation reactions at the tissue where the gene is delivered, is believed to be caused by a number of factors, including infection of viral vectors, and mutagenesis in normal cells into which genes are delivered [8]. One can anticipate that these toxicities are dose-dependent and that a E_{max} model for toxicity can be adequate. In the presence of observable dose-dependent toxicities, gene and gene delivery devices must be carefully engineered and dosed so that sufficient of effective genes are delivered to the target cells, while toxicities are minimized.

In conclusion, the present study applies a simple simulation model for targeted drug delivery to gene delivery, and shows that a simple pharmacokinetic assessment of the delivery device, the DTI, is directly related to pharmacodynamic outcome over a range of delivered doses, and so can be used to guide decision making during development of therapeutics.

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REFERENCES

[1] Hunt CA, MacGregor RD, Siegel RA. Engineering Targeted In Vivo Drug Delivery I. The Physiological and Physicochemical Principles Governing Opportunities and Limitations. Pharm Res 1986; 3(6):333-344.

[2] Tachibana R, Ide N, Shinohara Y, Harashima H, Hunt CA, Kiwada H. An assessment of relative transcriptional availability from nonviral vectors. Int J Pharm. 2004 Feb 11;270(1-2):315-21.

[3] Rowland M, McLachlan A. Pharmacokinetic considerations of regional administration and drug targeting: influence of site of input in target tissue and flux of binding protein. J Pharmacokinet Biopharm. 1996 Aug;24(4):369-87.

[4] Kamiya H, Akita H, Harashima H. Pharmacokinetic and pharmacodynamic considerations in gene therapy. Drug Discov Today. 2003 Nov 1;8(21):990-6.

[5] Jiang J, Yamato E, Miyazaki JI. Intravenous delivery of naked plasmid DNA for *in vivo* cytokine expression. Biochem. Biophys Res Comm. 2001 289: 1088-1092.

[6] Koh JJ, Kol KS, Lee M, Han S, Park JS and Kim SW. Degradable polymeric carrier for the delivery of IL-10 plasmid DNA to prevent autoimmune insulitis in NOD mice. Gene Therapy (2000) 7, 2099–2104

[7] Liu F, Qi H, Huang L, Liu D. Factors controlling the efficiency of cationic lipid-mediated transfection *in vivo* via intravenous administration. Gene Therapy (1997) 4, 517–523.

[8] Arguilar LK, Arguilar-Cordova E. Evoluation of a gene delivery clinical trial. Journal of Neuro-oncology. 2003; 65: 307-315.